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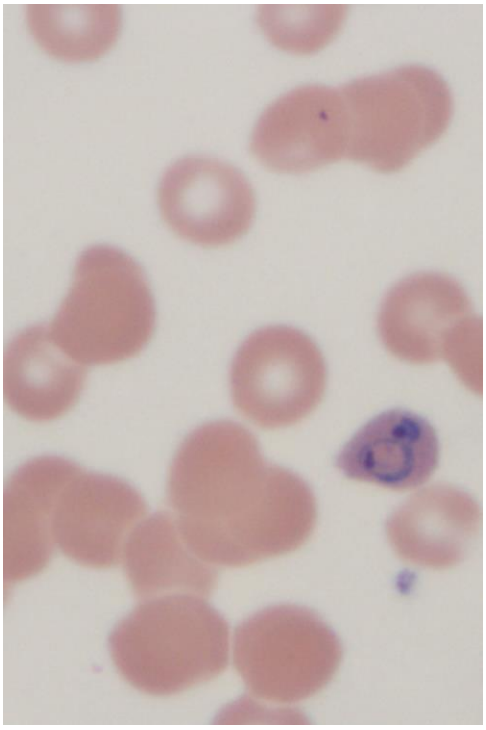
Is It Malaria?

Blood Borne Parasites That Cause Malaria Disease



1

Malaria

- 300-500 million cases per year worldwide
  - According to WHO 229 million cases occurred in 2019 and is attributed to 409,000 deaths.
  - Heaviest burdened region occurs in African
    - Children accounted for 78 % of all deaths
    - 90 % of all malaria deaths were seen in children under 5 years of age
- Half of the world's population lives in endemic areas

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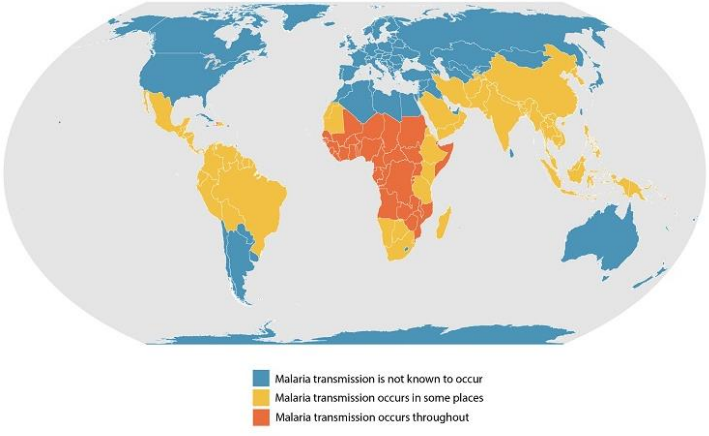
Malaria

- Approximately 1500-2000 cases reported every year in the United States
  - ➢Majority are imported
  - ➢Almost all in recent travelers
  - ➢Local transmission cases (rare)
  - ➢Blood transfusion cases (rare)
  - ➢Malaria causes 2 million deaths a year (mostly in Africa)
- 90-95% travelers will not become ill until they return home
- 85% develop symptomatic disease within 30 days of return

Geographic Distribution (CDC 2020)



This map shows an approximation of the parts of the world where malaria transmission occurs.

Concerns In Delay of Diagnosis

- 59% of malaria cases misdiagnosed
  - in nonendemic North American settings
- Average turnaround time from order to diagnosis ~2.5 days
- Estimated 80% of deaths preventable
  - Significant portion due to diagnostic delay or error

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Concerns In Delay of Diagnosis

Pregnant women have increased susceptibility to *P. falciparum*

- Highest susceptibility during 2nd and 3rd Trimester
- Early postpartum
- Severe complications

Possible mechanisms

- Sequestration of parasites in the placenta
- Depression of selected components of the immune system

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Concerns In Pathology

- *P. vivax* and *P. ovale* can develop dormant liver stages that can reactivate after asymptomatic intervals of up to 2 (*P. vivax*) and 4 years *(P. ovale*)

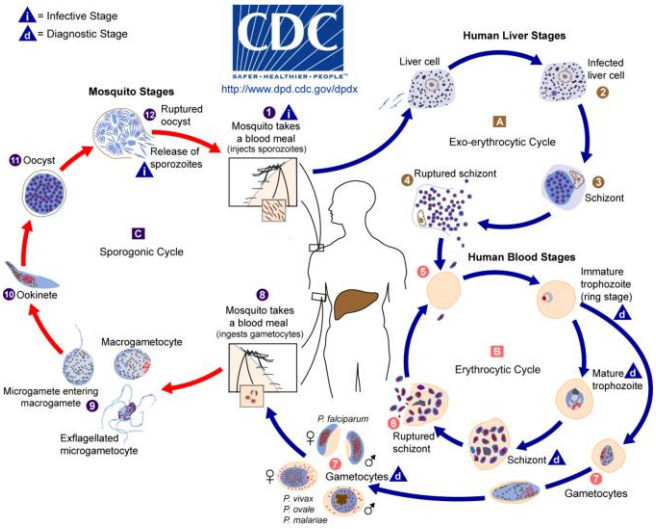
- An experienced laboratory technician or pathologist can distinguish between *P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale* based on the appearance of infected red blood cells and the parasites

Concerns In Pathology

- *P. knowlesi* can resemble either *P. falciparum* or *P. malariae* microscopically

- Two antimalarial drugs are derived from plants known to have medicinal value for centuries:
  - Artemisinin from the *Qinghaosu* plant (4th century China)
  - Quinine from the *Cinchona* tree (South America, 17th century)

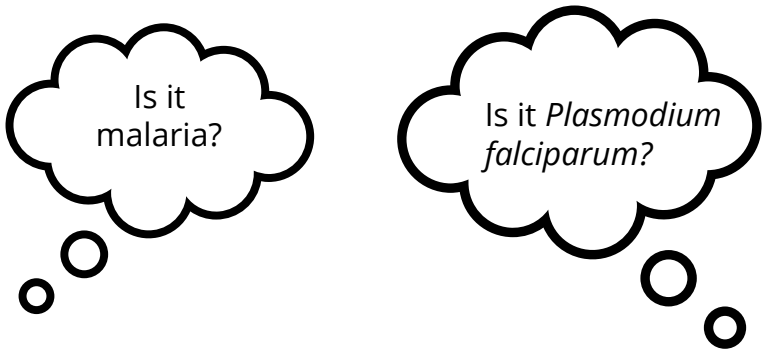
Plasmodium Life cycle



Malaria life cycle

- Gametocytes are a dead end in the human life cycle
  - Sexual reproduction occurs in the mosquito only
- Factors that affect development of Malaria parasites in mosquitos
  - Ambient temperature
  - Humidity

Two Major Questions



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Other General Considerations

- *P. falciparum* is potentially fatal
- *P. vivax and P. ovale* can sequester in Liver cells and require additional treatment to help prevent relapses
- *P. malariae* can clear spontaneously, or there may be recrudescence or a series of recrudescences over many years
- *Plasmodium species* are fairly vector dependent

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Clinical Features

- Incubation period is approximately 14 days before symptoms will first appear (prepatent phase)
- Symptoms usually begins with chills, followed by fever (fevers are caused by the schizont ruptures) ending with a stage of sweating (caused by the release of merozoites)
- Cycles occur periodically

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Malaria Cycles

- *P. falciparum:* Malignant tertian—indefinite but frequent (~48 hours)
- *P. vivax:* Benign tertian—48 hours
- *P. malariae:* Benign Quartan—72hours * the laid-back species of the malaria world
- *P. ovale :* usually less severe and tends to relapse less frequently
- *P. knowlesi* : regular 24-hour cycle

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Plasmodium falciparum

- Occurs anywhere malaria occurs
- Rings and/or gametocytes are usually only stages found in circulating blood
- Parasitemia can be high
  - more stages may be found in these circumstances
  - may not directly correlate peripheral blood parasitemia and severity of complications of immunologically naïve
- Invades all ages of red blood cells, number infected may exceed 50% *

Plasmodium falciparum

- Schizogony occurs in the spleen, liver and bone marrow rather than the circulating blood.
  - HRP antigen causes sequestration not allowing schizonts into the blood
- Ischemia due to the obstruction of blood vessels within organs produce various symptoms dependent on organ involvement
- Symptoms typically appear 8-12 days after infection
- Quantification very important in cases of high parasitemia
  - Transfusion therapy may be needed

Plasmodium falciparum

- Causes cytoadherence associated with severe malaria
- Black Water Fever
- DIC
- Cerebral malaria is the most serious complication and a major cause of death in *P. fal* patients. It occurs in up to 10% of the *P. fal* patients
- Recrudescence and relapses are rare except in cases of treatment failure

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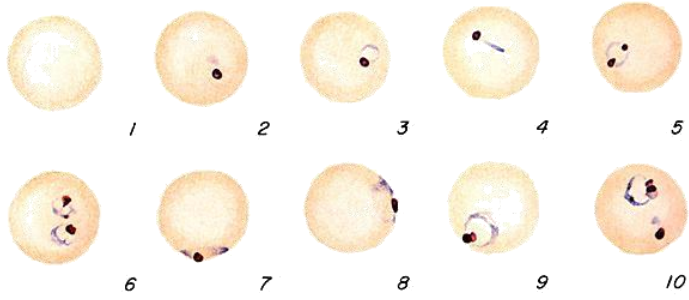
Plasmodium falciparum Ring forms

- Thin and delicate appearing cytoplasm
- Multiple rings may be found in RBCs (*not exclusive to *P. fal*)
- Double chromatin dots (*not exclusive to *P. fal*)
- Applique forms may be found (acole)



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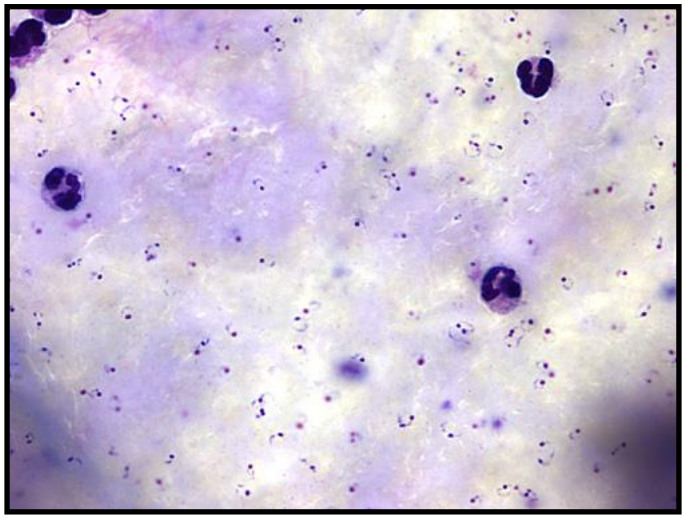
Plasmodium falciparum Thick Film



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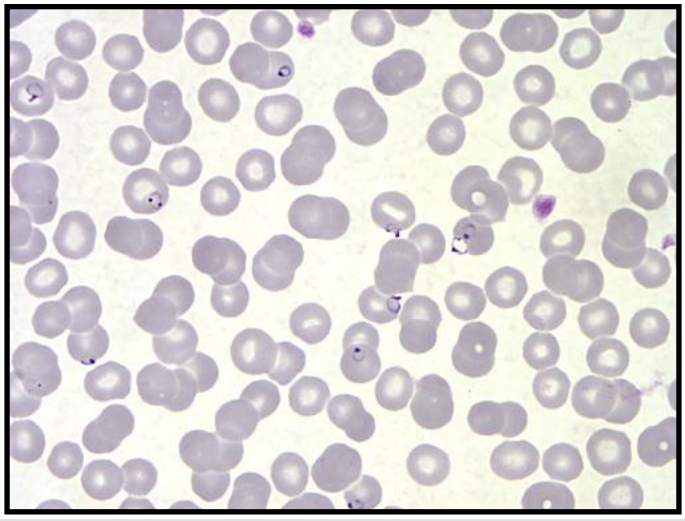
Plasmodium falciparum Thin Film



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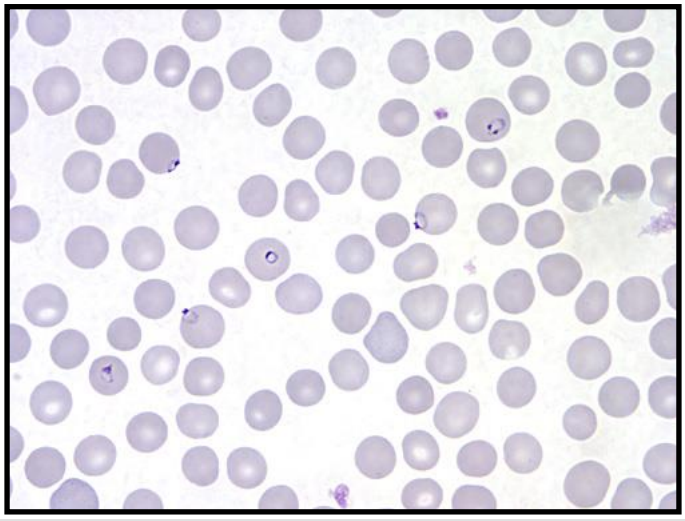
Plasmodium falciparum Thin Film



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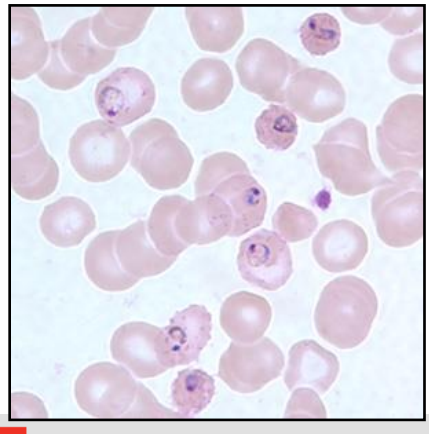
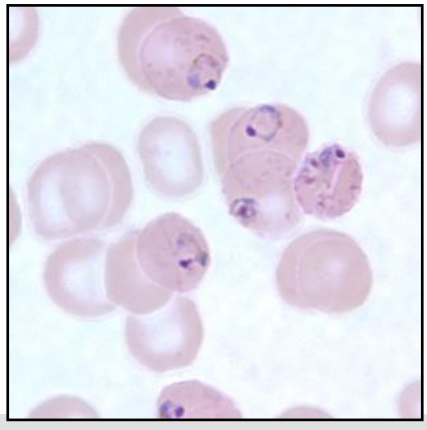
Plasmodium falciparum Maurer's clefts

- Looks like chocolate chunk cookies (cytoplasmic dots)
- Usually found in finger stick preps
- Fewer and larger than the cytoplasmic stippling of *P.ovale/P.vivax*





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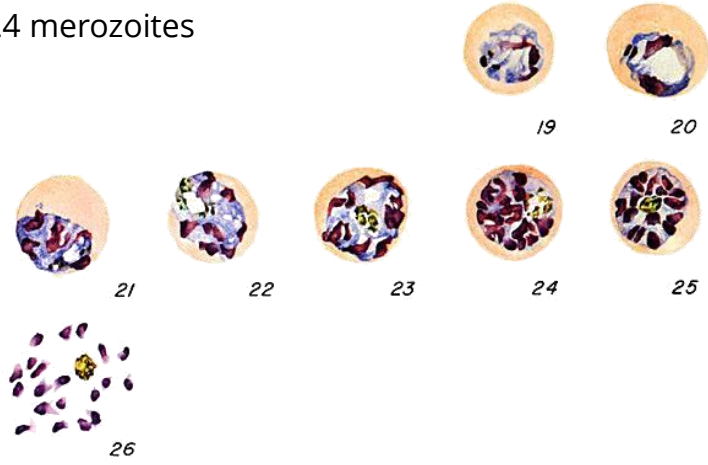
Plasmodium falciparum Schizonts

- Rarely seen in blood stream of *P. falciparum* infections
- 8-24 merozoites



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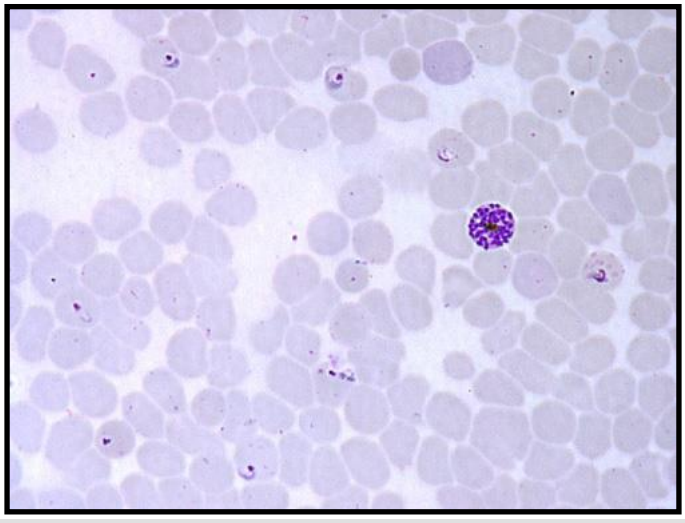
Plasmodium falciparum Schizont



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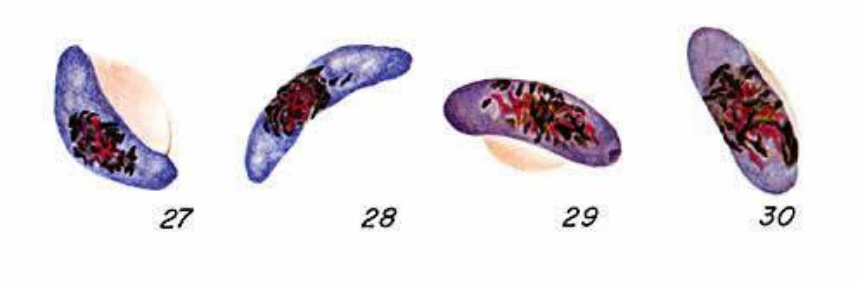
Plasmodium falciparum Gametocytes

- Typically crescent shaped with pigment (banana, sausage)
- Laveran's bib-Remnants of host RBC seen during the gametocyte stage



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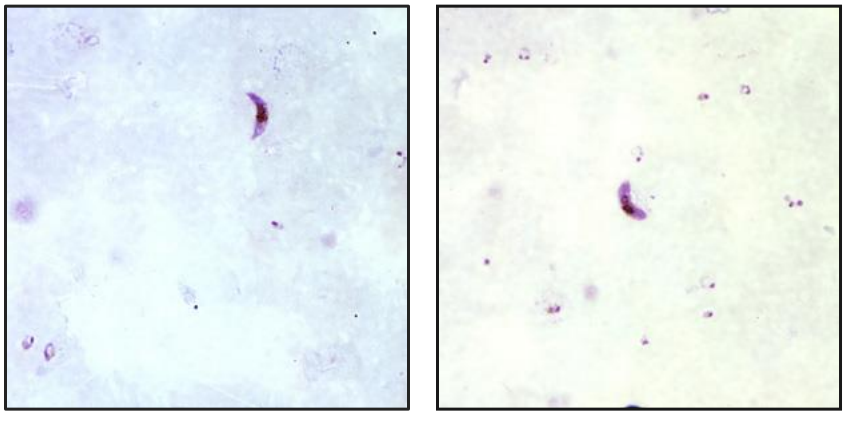
Plasmodium falciparum Thick Film



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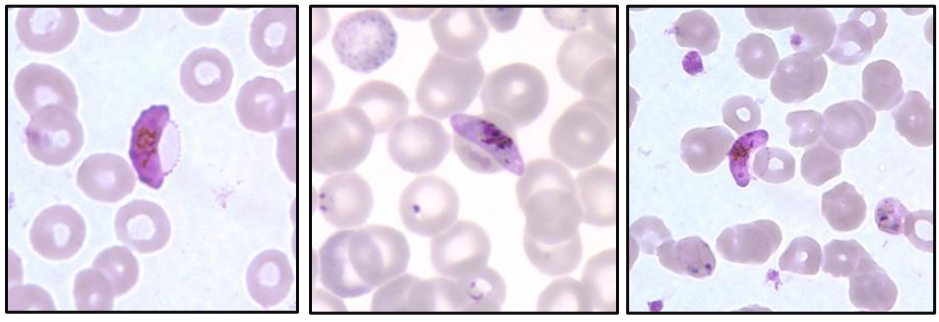
Plasmodium falciparum Thin Films



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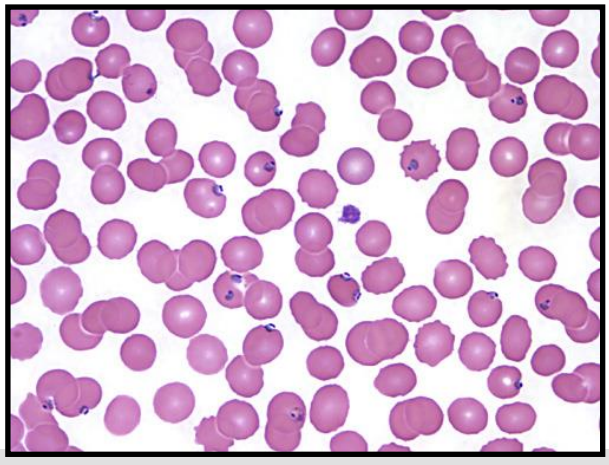
Atypical presentation of *P. falciparum* trophozoites

- Occurs when blood smears are not made in a timely manner
- Blood at room temperature begins the sexual stages of the lifecycle



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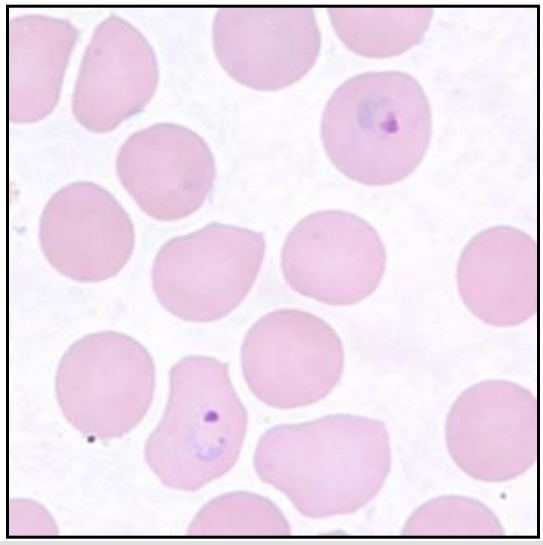
Plasmodium vivax



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Plasmodium vivax

- Infects only the reticulocytes of the blood
- Parasitemia is limited to only 2-5 % of the available cell
  - size of infected RBC's up to 2X that of a normal RBC
- All stages are found in the blood stream
- Trophozoites can be amoeboid in appearance
- Schüffners dots can be seen under the proper staining (Giemsa)

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Plasmodium vivax

- Splenomegaly occurs during the first few weeks of infection
  - size of spleen will return to normal if treated during the early phases
- Secondary or dormant schizogony (hypnozoites)
- Irregular periodicity followed by establishment of 48-hour cycles
- Amoeboid rings and mature schizonts with 12-24 merozoites
- Gametocytes are usually large and round

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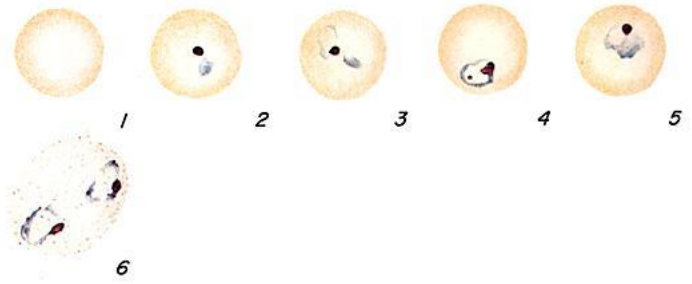
Plasmodium vivax Ring Forms

- Usually thicker with a single larger chromatin dot.
- Usually one parasite per host cell, but multiples can be seen



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Plasmodium vivax Symptomology

- First exposure: headache, photophobia, muscle aches anorexia nausea and sometimes vomiting occurs before symptoms appear.
- In patients that have prior exposure, the parasites can be found in the blood stream days before symptoms appear.

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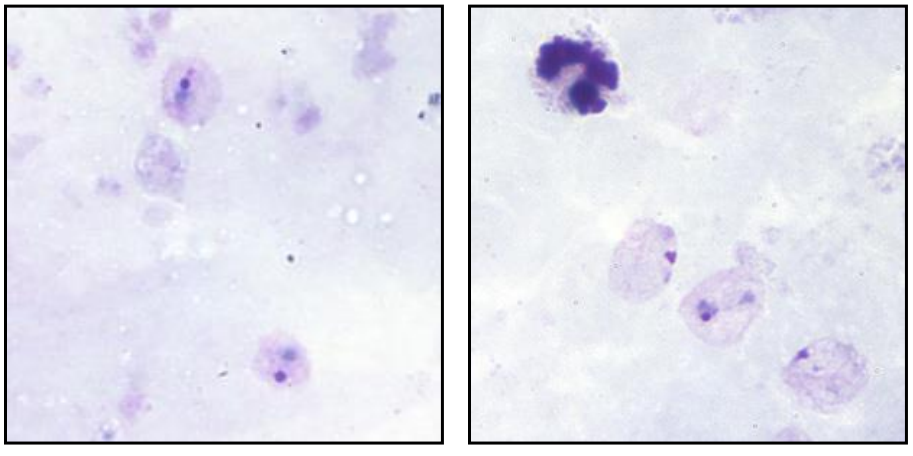
Plasmodium vivax Rings in thick films



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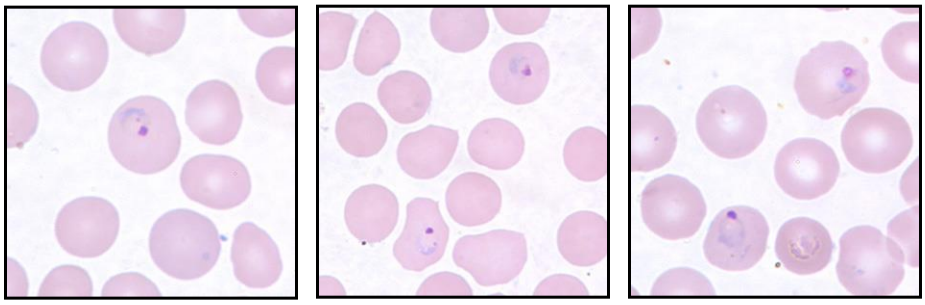
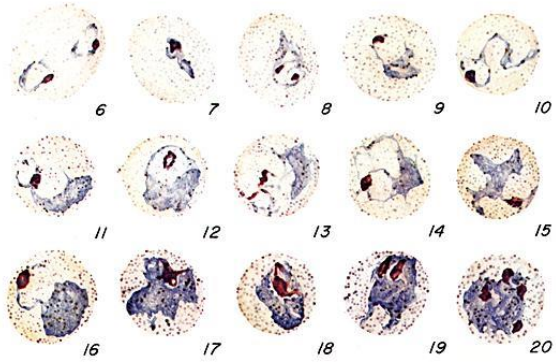
Plasmodium vivax: Rings in thin films

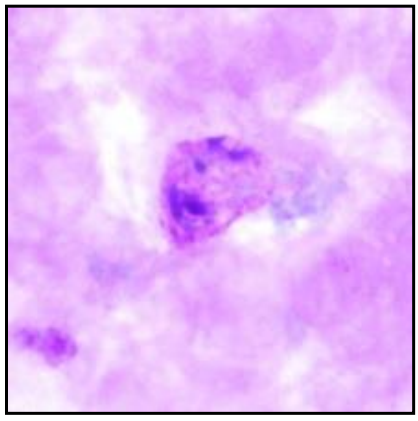


Plasmodium vivax Trophozoites

- Usually amoeboid in appearance.
- Infected RBC's (1.5 times the size of normal RBC)
- Schüffners dots may be present.
- Band forms are present as well.(Very large with fine pigment)



Plasmodium vivax Trophozoite thick smear



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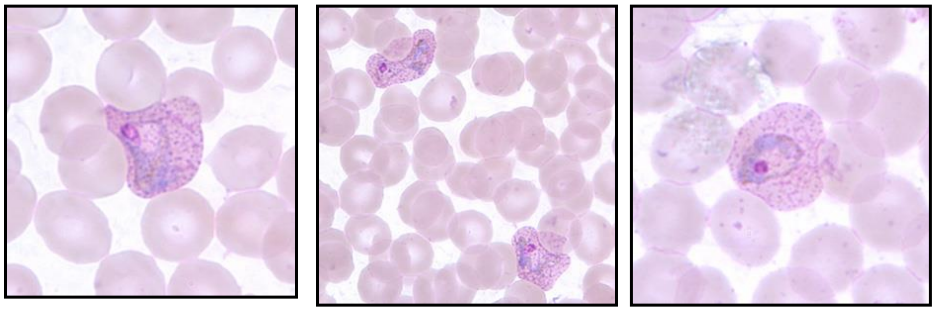
Plasmodium vivax trophozoites, thin smears (Giemsa Stain)



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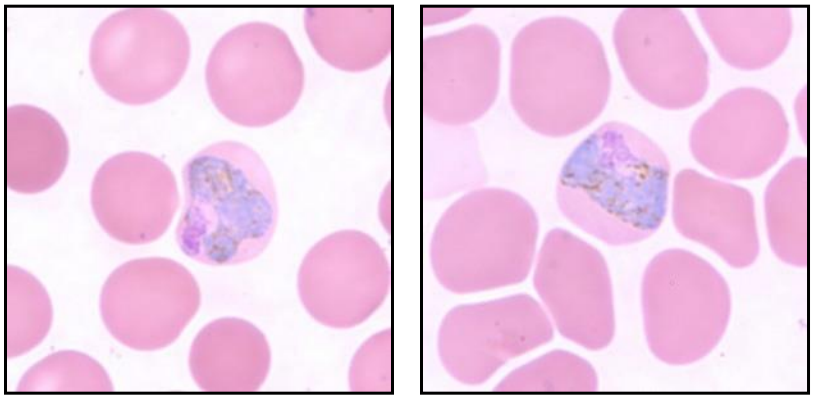
Plasmodium vivax trophozoites, thin smears (Wright's Stain)



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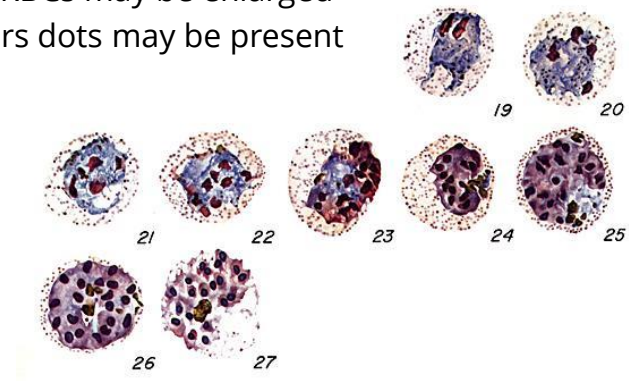
Plasmodium vivax Schizonts

- >13 (16-24) merozoites
- Mature schizonts have coalesced pigment
- Infected RBCs may be enlarged
- Schüffners dots may be present



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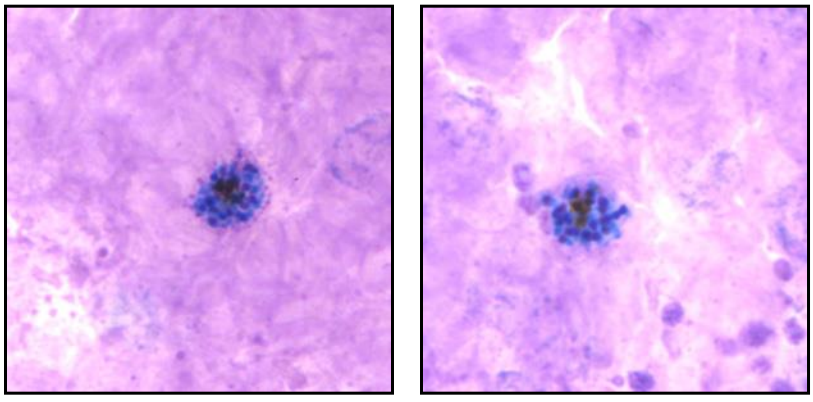
Plasmodium vivax Schizonts in thick smears



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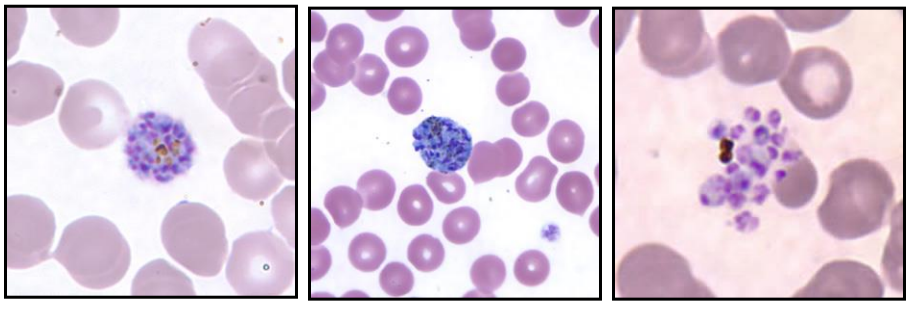
Plasmodium vivax: Schizonts in thin smears



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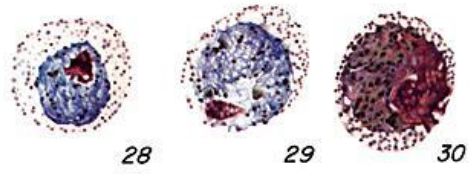
Plasmodium vivax Gametocytes

- Mature gametocytes enlarged to 2x normal RBC
- Round to oval and usually fill host RBC
- Pigment is usually fine and evenly dispersed
- Schüffner's dots may be seen



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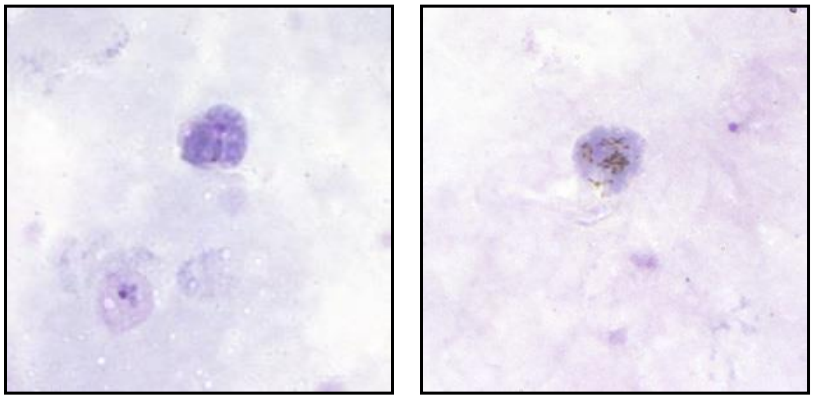
Plasmodium vivax Gametocytes thick smears



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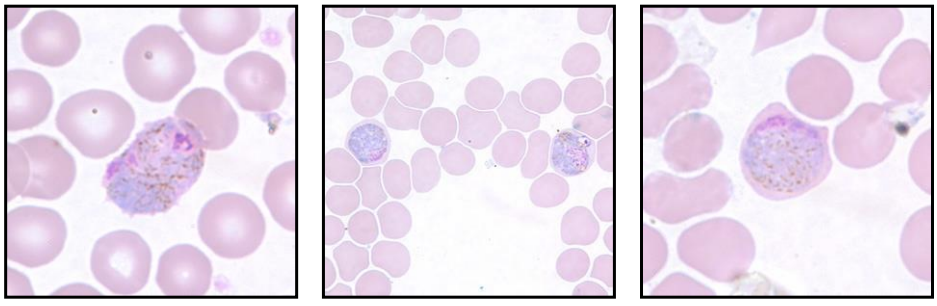
Plasmodium vivax Gametocytes thin smears



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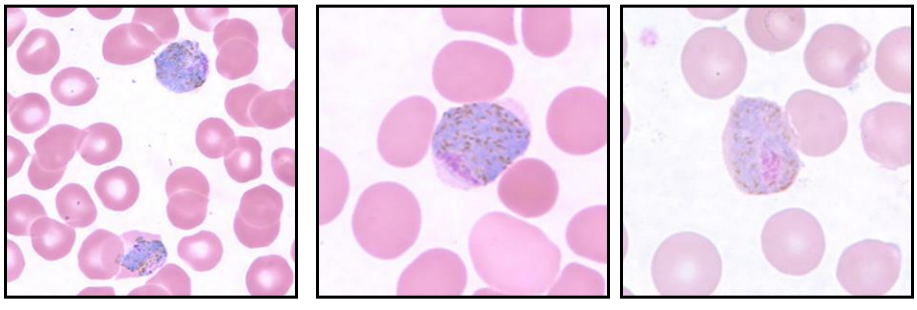
Plasmodium vivax Gametocytes thin smears



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Plasmodium ovale

P. ovale and *P. vivax* infections are similar though *P. ovale* malaria is usually less severe

- Fewer less frequent relapses usually ending with spontaneous recovery
- Only infects the reticulocytes
- In the past thought to undergo a second schizogony , but newer findings indicate that hypnozoites have not been demonstrated to do so in biologic experiments

Plasmodium ovale

- After a few days of irregular periodicity a regular 48-hour pattern is established
- Schüffners dots present in enlarged RBC's (Young cells) from the beginning of the life cycle
- Developing rings are more amoeboid than *P. vivax* and mature schizonts contain an average of 8 merozoites
- Incubation period is similar to *P. vivax* but symptom severity is much less

Plasmodium ovale

- Geographic range: Tropical Africa, the middle east, Papua New Guinea and Irian Jaya in Indonesia
- In southeast Asia, *P. Ovale* infections may cause benign relapsing malarias
- In both Southeast Asia and Africa two different types of *P. ovale* circulate in humans
- Human infections with variant types of *P. ovale* are associated with a higher parasitemia

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Plasmodium ovale Rings

- Usually thicker with a single chromatin dot (may be double)
- Often one parasite per infected RBC, but multiply-infected RBCs not uncommon
- Schüffner's dots may be present

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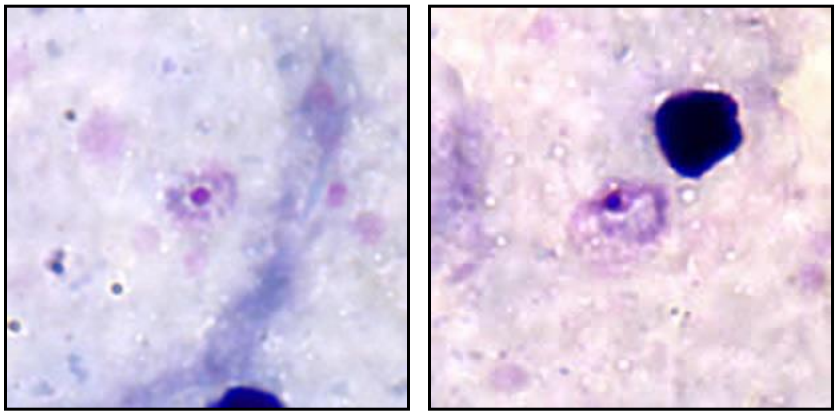
Plasmodium ovale Rings, thick smears



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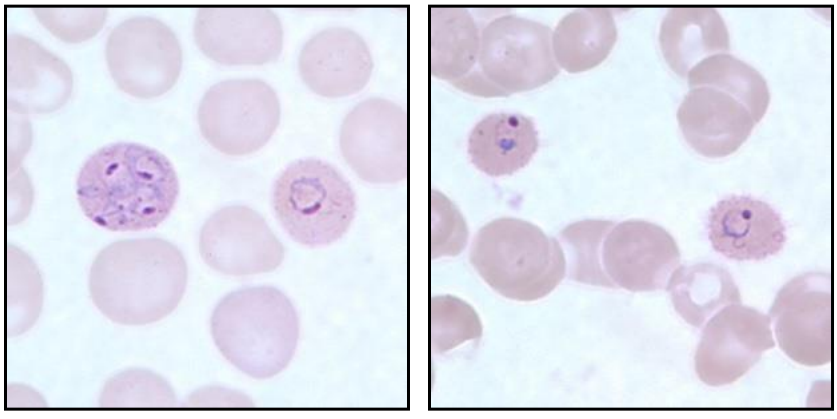
Plasmodium ovale Rings, thin smears



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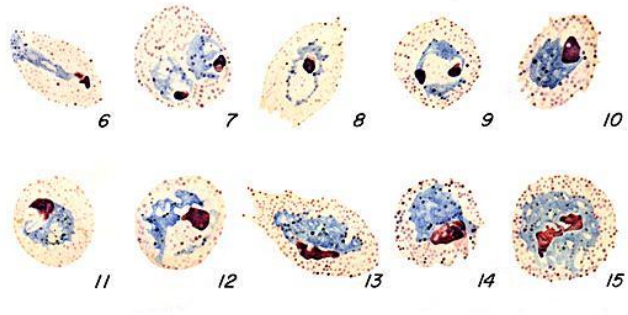
Plasmodium ovale Trophozoites

- Trophozoites may start to become amoeboid, but not to the degree as *P. vivax*
- Schüffner's dots may be seen
- Enlarged, but usually not as much as with *P. vivax*
- Elongation and fimbriation common (note difference from crenation)



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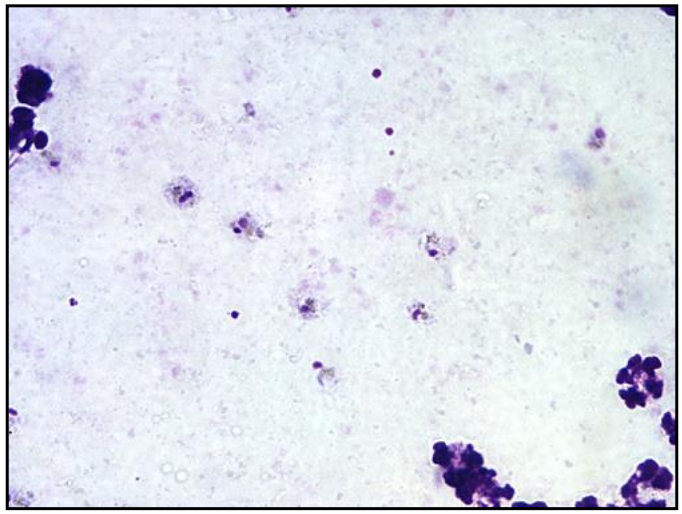
Plasmodium ovale Trophozoites thick smear



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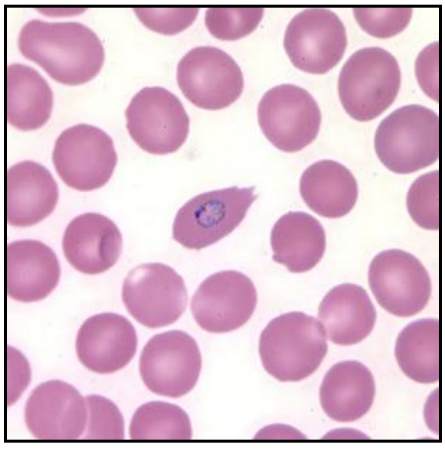
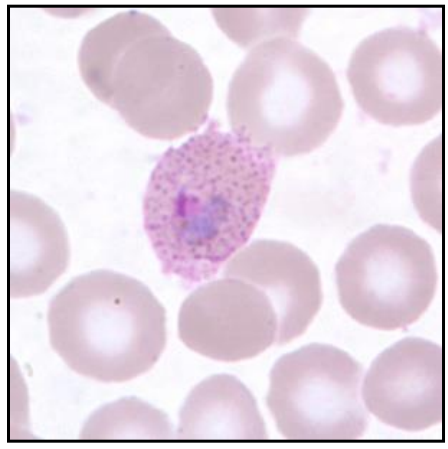
Plasmodium ovale Trophozoites, thin smears





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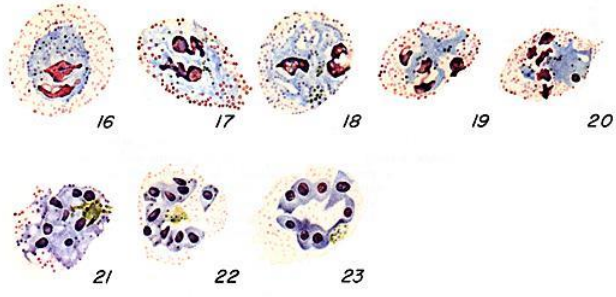
Plasmodium ovale Schizonts

- Mature schizonts usually have <13 merozoites (6-16)
- Schüffner's dots may be seen
- Enlarged, but usually not as much as with *P. vivax*
- Elongation and fimbriation can occur (note difference from crenation), but may also be rounded



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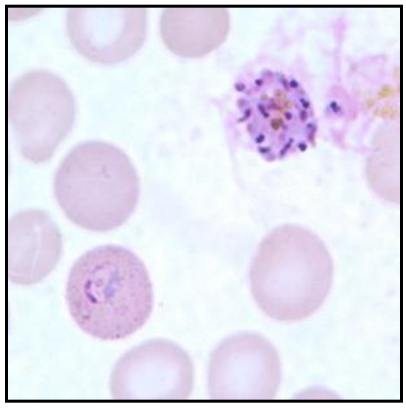
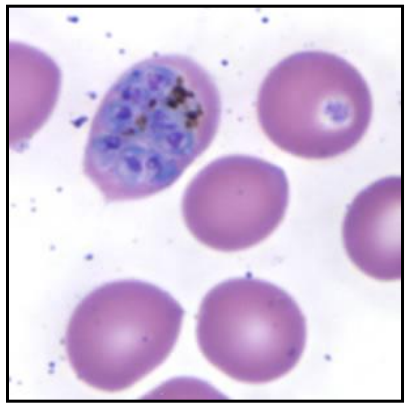
Plasmodium ovale Schizonts





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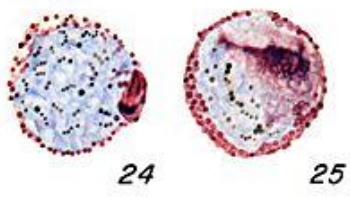
Plasmodium ovale Gametocytes

- Enlarged, but not usually as big as *P. vivax* (1-1.25x normal RBC)
- Pigment usually more coarse than *P. vivax*
- Schüffner's dots may be seen
- Usually rounded (may be elongated and fimbriated, especially in immature gametocytes)



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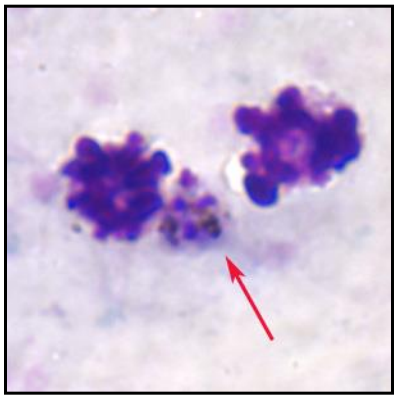
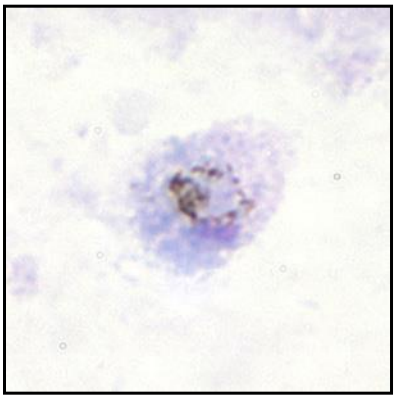
Plasmodium ovale Gametocytes, thick smears





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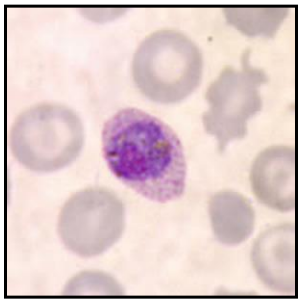
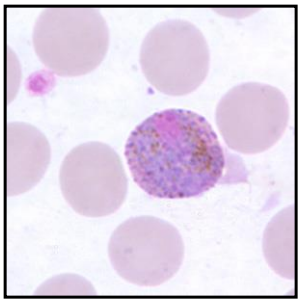
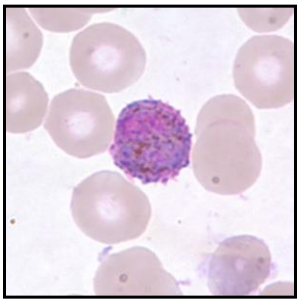
Plasmodium ovale Gametocytes, thin smears







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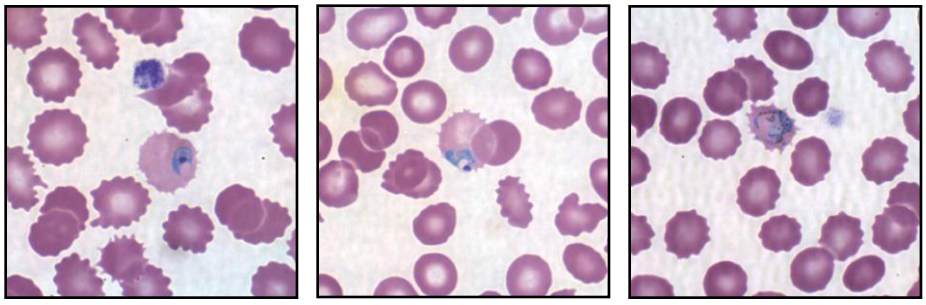
Crenation (not fimbriation)



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Plasmodium malariae

- Quartan malaria
- Invades primarily older cells
- Incubation period between infection and symptoms is much longer than *p.vivax/ovale* (27-40 days)
- Regular periodicity of 72 hours from the beginning with more severe paroxysms including a longer cold stage and more severe symptoms during the hot stage

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Plasmodium malariae

- No true stippling
- RBC's may have fimbriated edges
- Developing rings tend to demonstrate band forms
- Mature schizonts contain an average of 6-12 merozoites
- Infected RBC's tend to be normal to small (old RBC's)

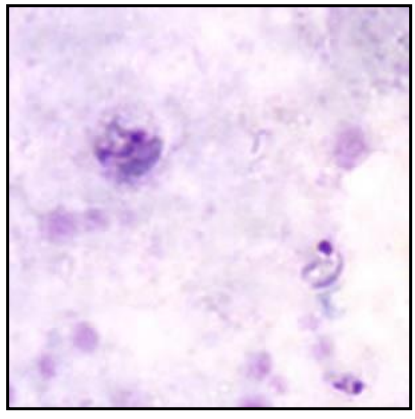
Pathogenesis and spectrum of disease

- Proteinuria is common and associated with clinical signs of nephrotic syndrome
- Deposits of antigen antibody complexes within the glomeruli
  - Glomerulonephritis is the most common lesion seen in quartan malaria
- Chronic glomerulonephritis associated with P. malariae is usually not reversible with therapy
  - Genetic and environmental factors may play a role in disease as well

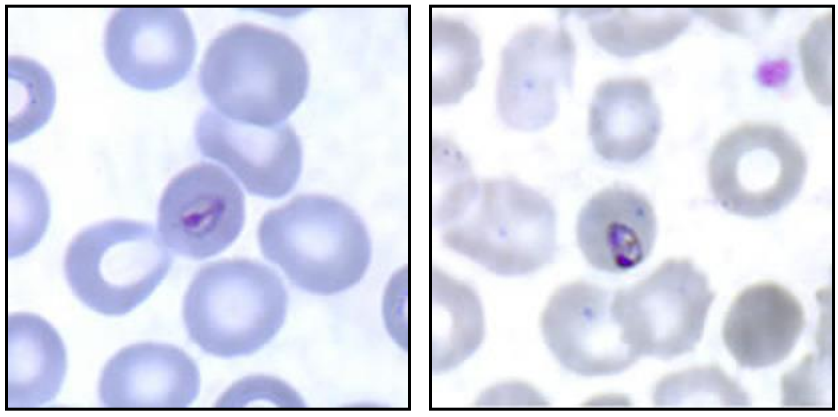
Plasmodium malariae Rings

- Rings usually small
- Often one, sometimes two, chromatin dots
- May see 'bird's eye' forms

Plasmodium malariae Rings, thick smear

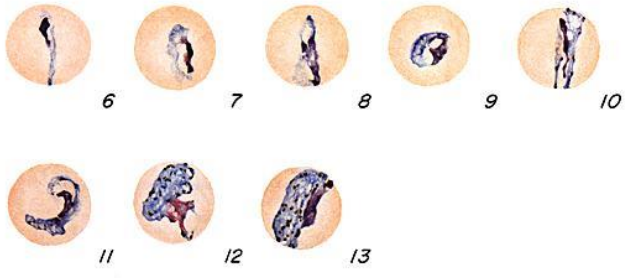


Plasmodium malariae Rings, thin smear

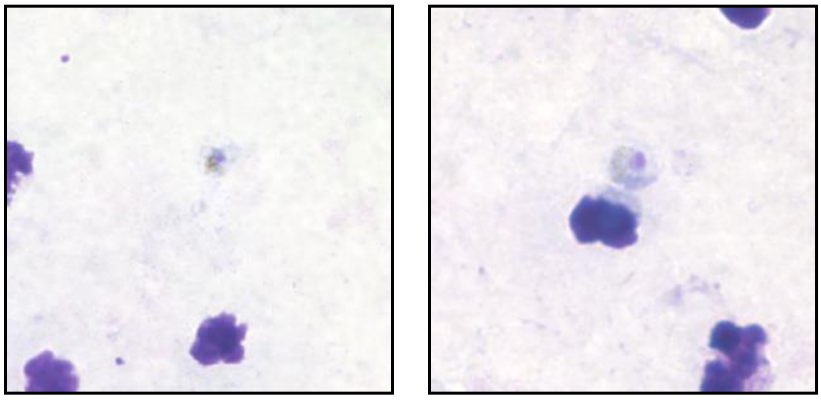


Plasmodium malariae Trophozoites

- Elongate as they develop.
- May see 'basket' or 'band' forms.



Plasmodium malariae Trophozoites, thick smear



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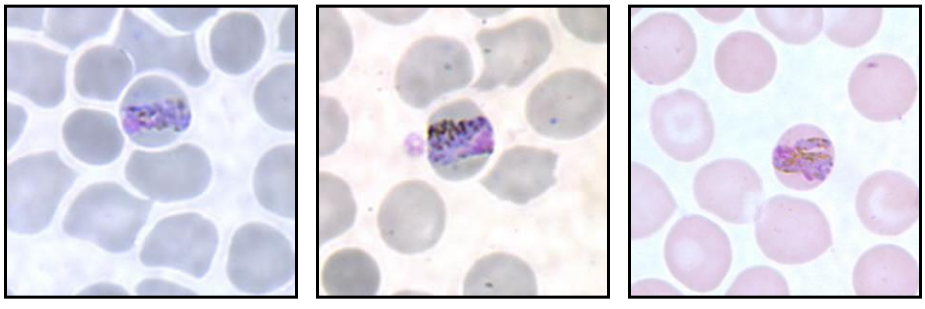
Plasmodium malariae Trophozoites, thin smears (band forms)



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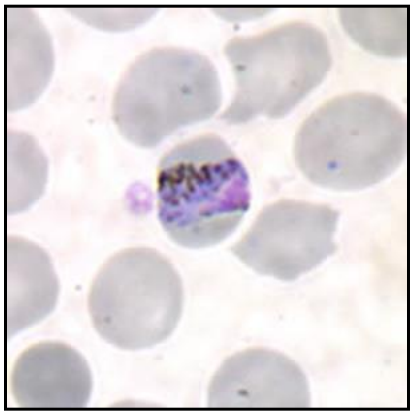
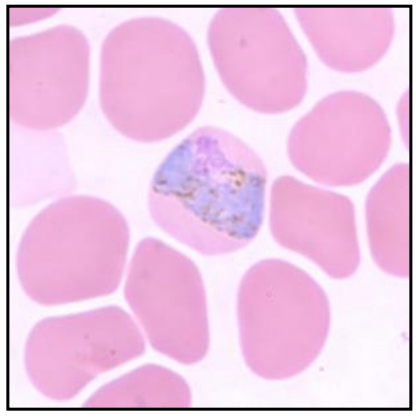
Band Forms *P. malariae vs P. vivax*



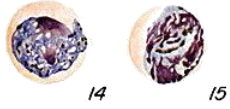
P. malariae

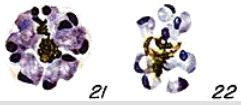


P. vivax

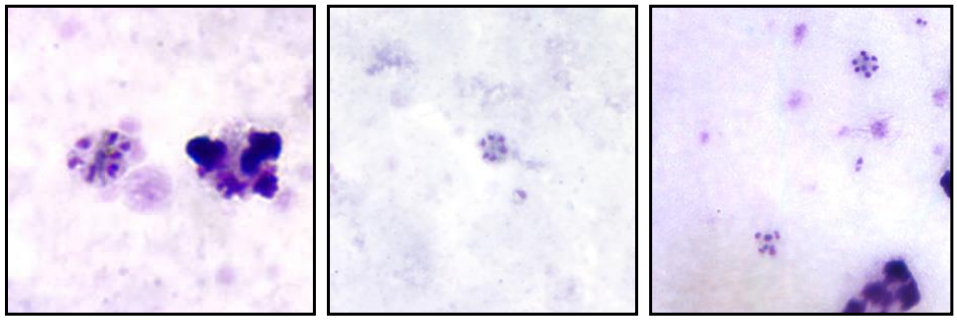
Plasmodium malariae Schizonts

- Mature schizonts have 6-12 merozoites
- Often in rosette-shaped patterns
- Pigment usually coalesced and centrally-located
- May be smaller than host RBC





Plasmodium malariae Schizonts, thick smears



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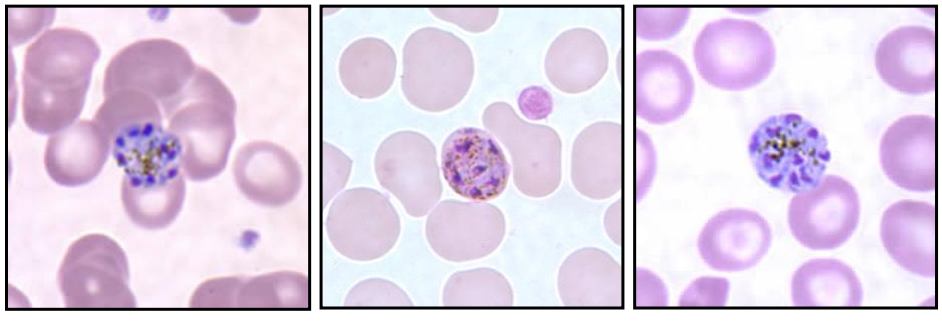
Plasmodium malariae Schizonts, thin smears



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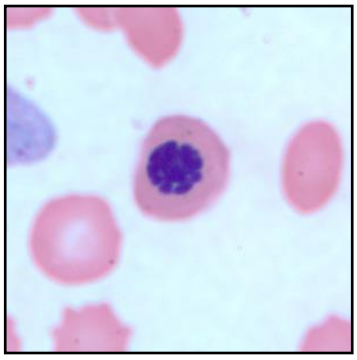
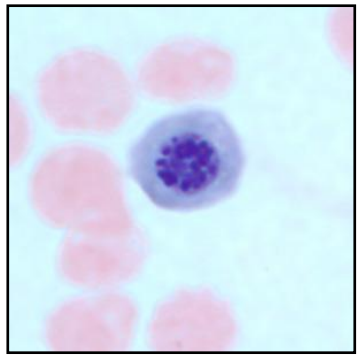
Nucleated Red Blood Cells (not Schizonts)

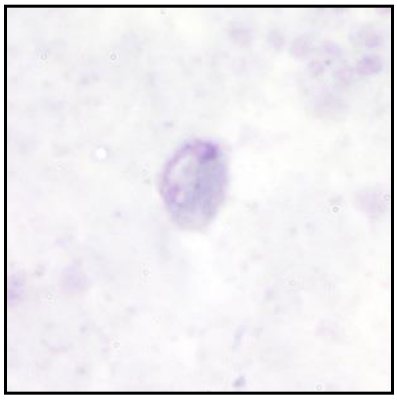


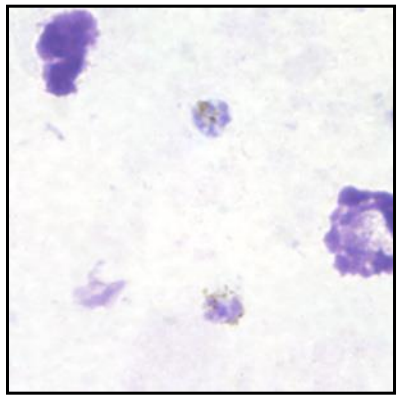


Plasmodium malariae Gametocytes

- Round in shape
- May be smaller than normal RBC
- Pigment usually coarse

Plasmodium malariae Gametocytes, thick smear





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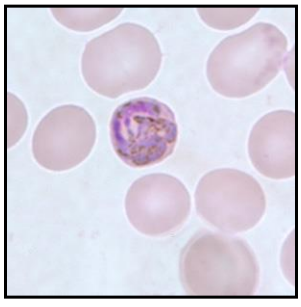
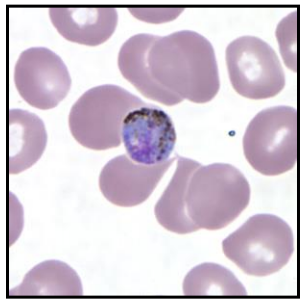
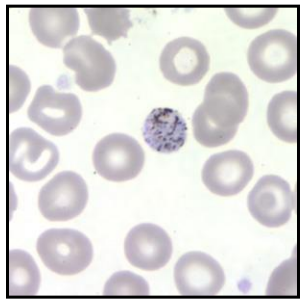
Plasmodium malariae Gametocytes, thin smears







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Plasmodium knowlesi

- Simian malariae, the fifth human malaria
- Is prevalent among Crab-eating macaques (monkey)
- *P. knowlesi* infection should be considered in patients with travel history to forested areas of southeast Asia especially if:
  - *P. malariae* is diagnosed
  - Unusual forms are seen upon microscopy
  - Mixed infection with *P. falciparum/ P. malariae* is diagnosed
- Disease is potentially fatal

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Plasmodium knowlesi

- Regular 24-hour cycle
- Invades all ages of RBC's
- Detection of mixed infections can be quite difficult
- No true stippling
- Multiple rings per RBC, band forms and mature schizonts with 16 merozoites.

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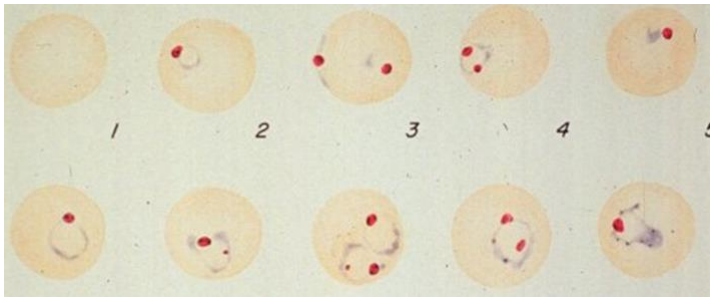
Plasmodium knowlesi Ring stages

- Early stages resemble *Plasmodium falciparum*
- Multiple rings per infected RBC



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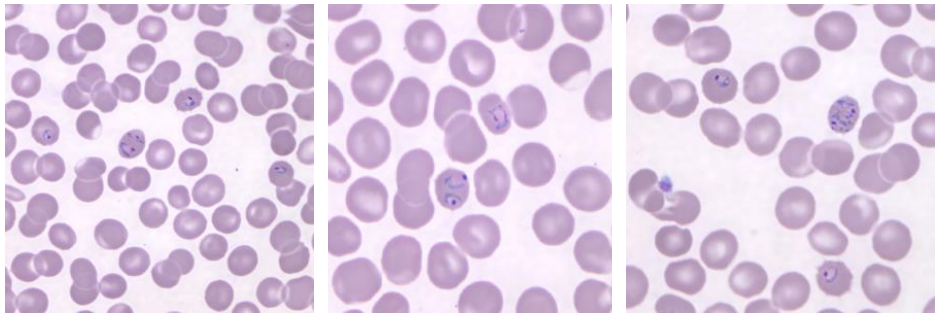
Plasmodium Knowlesi Ring forms thin smears



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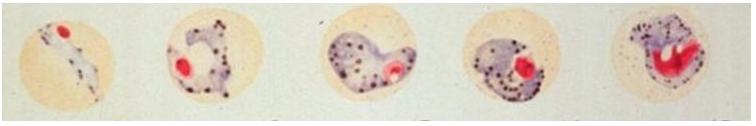
Plasmodium knowlesi trophozoites

- Developing trophozoites resemble *P. malariae*
- Vacuole is lost during maturation
- Parasite becomes smaller and more compact
- Stippling "Sinton and Mulligan's"



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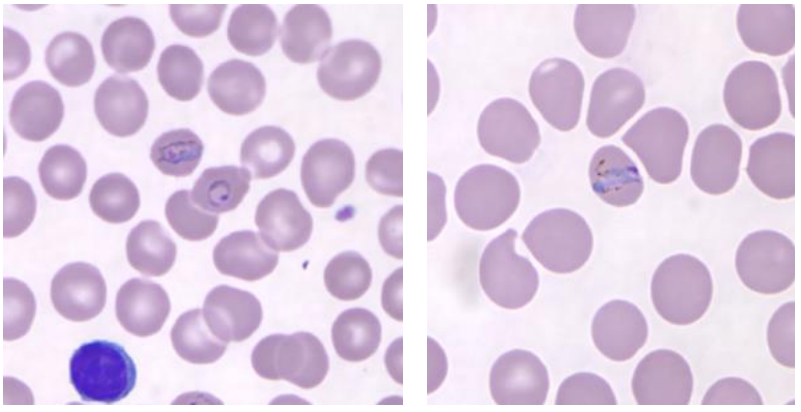
Plasmodium knowlesi trophozoites, thin smear



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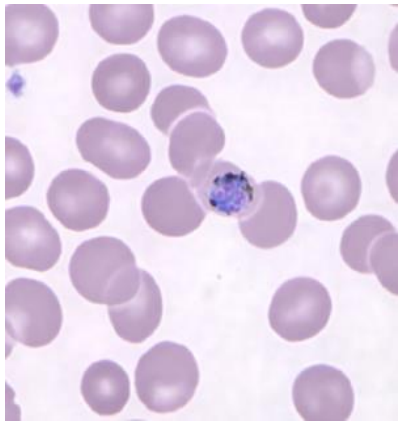
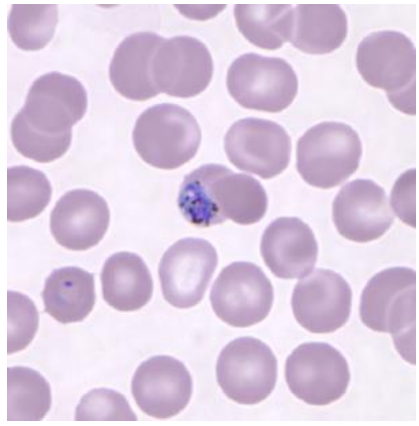
Plasmodium knowlesi Gametocytes





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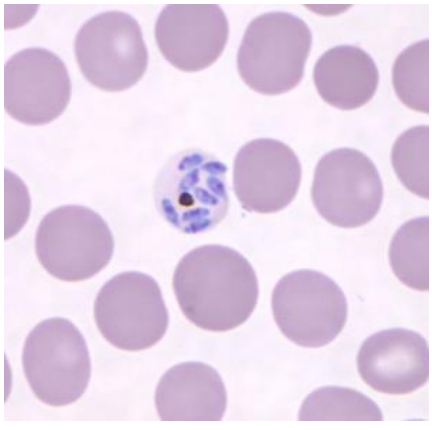
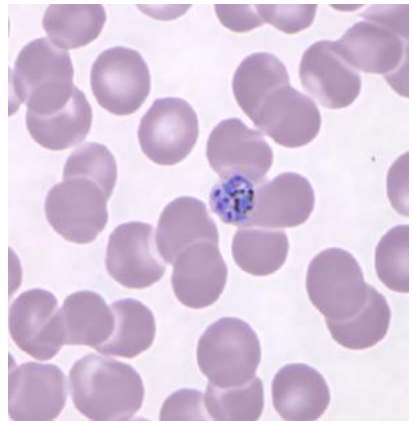
Plasmodium knowlesi Schizonts





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Babesia species

- Includes about 100 species transmitted by deer ticks of the genus *Ixodes*
- Worldwide distribution
- 2 species cause the most human infections.
  - *Babesia microti* is the cause of most human infections in the United States.
  - *Babesia divergens* tends to be more common in Europe and often found in splenectomized patients
- Several outbreaks in humans have been recorded in the Northeastern United States
- Life cycle is similar to *plasmodium* species, no exoerythrocytic stage has been described

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Babesia species Pathogenesis

- *Babesia* is clinically similar to malaria with symptoms including high fever, myalgias,malaise, fatigue hepatosplenomegaly and anemia
- Cases of *Babesia* in the US usually occur in nonsplenectomized invidividuals with mild symptoms
- Mild cases can resolve spontaneously while more serious cases require drug therapy

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Babesia species

- Sporozoites injected by the bite of an infected tick invade erythrocytes directly
- Trophozoites reproduce by binary fission rather than schizogony
- Trophozoites can mimic the rings seen with *P. falciparum* , however there are differences that will help differentiate the two parasites
- Species level Identification by PCR

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Babesia species

- Extraerythrocytic and intraerythrocytic forms may be found
- Morphologically resemble ring forms of *Plasmodium* spp
- Erythrocytic forms may be pyriform, oval, or round, and often are ameboid or vacuolated
- More than one parasite can be found in a single RBC

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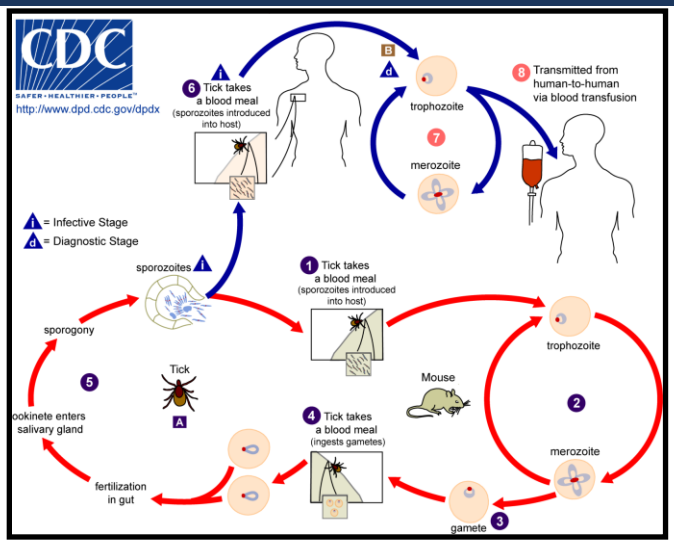
Life Cycle of *Babesia* species



Babesia species

- Pigment is **not** present in the organisms
- There is no enlargement of the RBC
- No gametocytes are found in human infections
- In some species, tetrads are formed as a result of fission

Babesia species

- *Babesia* trophozoites range from 1 to 5 µm; the smallest are smaller than P. falciparum rings
- Ring forms outside of the RBC's
- 2 to 3 rings per RBC common
- Ring forms are more pleomorphic and range with size even within an RBC
- Diagnostic tetrads Maltese Cross may be present.

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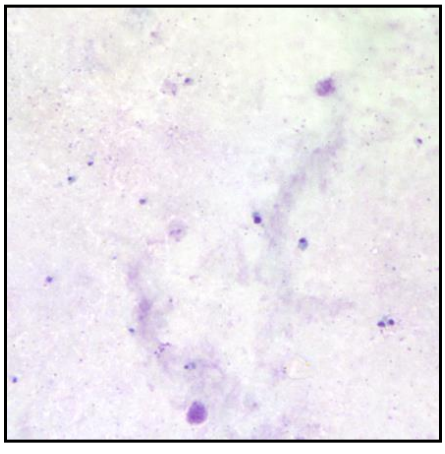
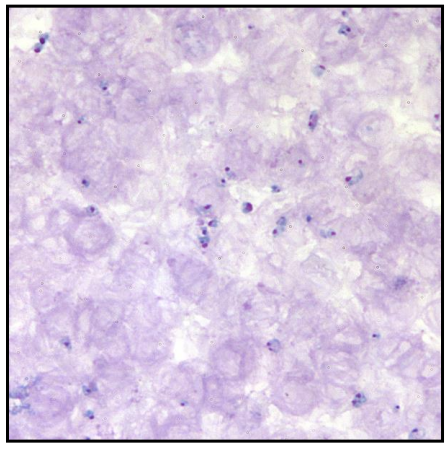
Babesia species in thick smears





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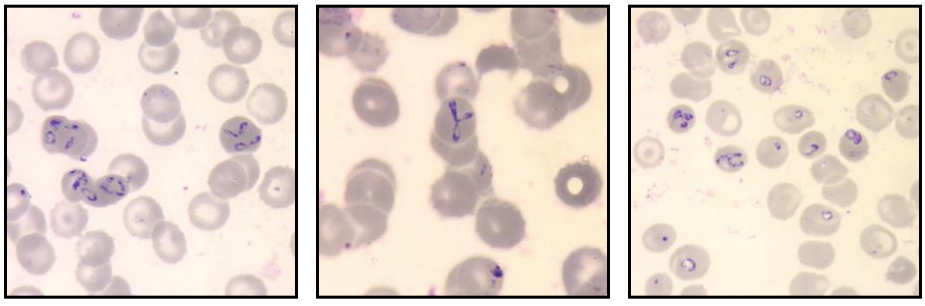
Babesia in thin smears



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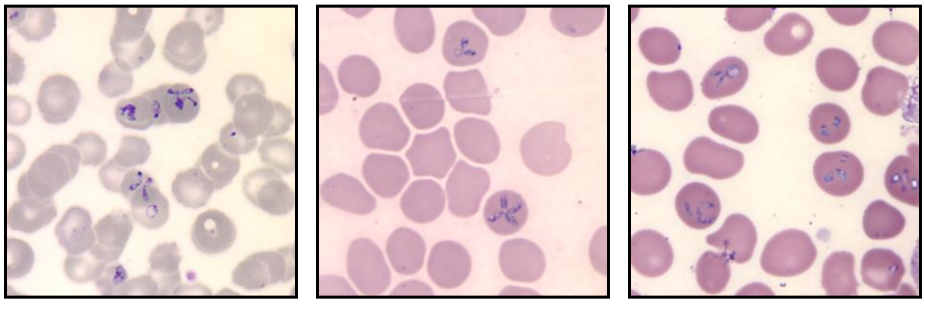
Babesia in thin smears



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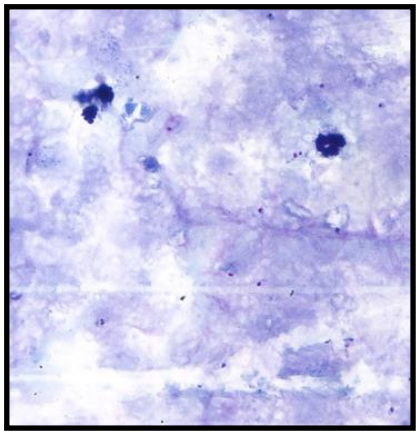
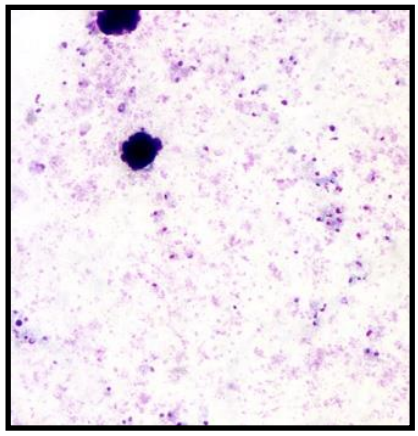
Plasmodium falciparum vs *Babesia* Thick Smears



Plasmodium falciparum



Babesia microti

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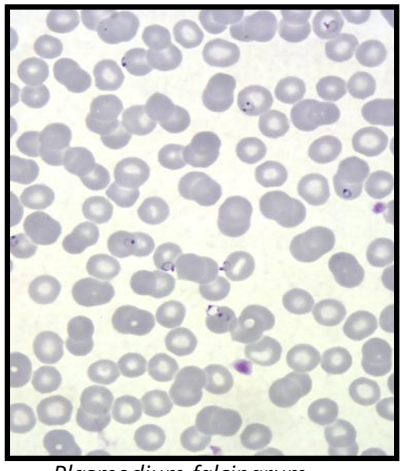
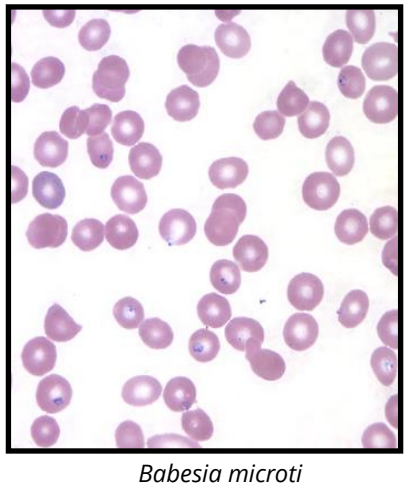
Plasmodium falciparum vs *Babesia* Thin smears



Plasmodium falciparum



Babesia microti

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Quantification of Parasites

- In some cases, quantification of parasites yields clinically useful information. Malaria parasites can be quantified against blood elements such as RBC's or WBC's.

- To quantify malaria parasites against RBC: count infected RBC's among 500-2,000 RBCs on the thin smear and express the results as % parasitemia.

% parasitemia= (Infected RBC's /Total RBC's) x 100

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Thank You

To receive a certificate of completion, please complete the post-course examination.

Questions? Please email LabTraining.Health@tn.gov

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